Original Research Article

Comparative evaluation different laboratory methods for detection of methicillin resistant staphylococcus aureus

Prabhat Kumar Ranjan[1], Mukesh Kumar[2] , Priti Singh[3*], Ratnesh Kumar[4], S N Singh[5]

[1,2,3] Tutor, Department of Microbiology, Patna Medical College and Hospital, Patna, India
[4]Assistant Professor, Department of Microbiology, Patna Medical College and Hospital, Patna, India
[5]Professor & HOD, Department of Microbiology, Patna Medical College and Hospital, Patna, India

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Abstract

Background: MRSA is any strain of Staphylococcus aureus that has developed resistance to beta-lactamase antibiotics which include Penicillin and Cephalosporins. With the increasing prevalence of MRSA infections, it has become important to have an ideal method of detection. other than the molecular/MIC so that it can be carried out even in the remote areas. **Aim:** To compare the three conventional methods namely oxacillin screen agar, cefoxitin disk diffusion and oxacillin disk diffusion for the detection of MRSA, with the gold standard MIC determination. **Materials and Methods:** This study was conducted at Department of Microbiology, at Patna Medical College and Hospital, Patna from March 2020 to September 2020. 75 strains of Staphylococcus aureus were included in the study, confirmed by slide and tube coagulase tests. Oxacillin screen agar: By direct colony suspension method, any growth considered as resistant to methicillin. Oxacillin disk diffusion:After performing the test, a Zone of inhibition< 13, the strain is resistant. Cefoxitin disk diffusion:A 30 microg disk was used zone of inhibition < 20 is resistant. Minimum inhibitory concentration determination: Agar dilution method was used to determine the Minimum Inhibitory Concentration (MIC). Organisms with MIC of 4 and above were considered as MRSA and those with MIC below 4 were considered MSSA. **Results:** The results as obtained by all the three methods were observed to be the same. Out of the 75 strains of Staphylococcus aureus, all the methods detected 19 MSSA (25%) and 56 (75%) MRSA. All the three methods showed 100 percent sensitivity and specificity. **Conclusion:** Since all the tests showed 100 percent sensitivity and specificity we recommend that any two of the methods be used combined for accuracy and reliability.

Keywords: MRSA; MIC; Oxacillin; Cefoxitin.

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Introduction

Staphylococcus aureus is gram positive, non-motile cocci, often found in grape like clusters and has continued to be a major source of human infection since ages. Penicillin was nothing less than a super drug since its discovery by Alexander Fleming and proved to be extremely effective in treatment of staphylococcal infections. However, Over time and use, the Staph bacteria naturally developed a resistance to the drug, primarily due to the adaptive nature of the bacteria, the rampant overuse of the antibiotics in their early stages and the emergence of beta-lactamase resistance. Since the first report of methicillin-resistant Staphylococcus aureus (MRSA) as a major nosocomial pathogen in the 1960s, the incidence of infections caused by this organism continues to rise[1]. MRSA is any strain of Staphylococcus aureus that has developed resistance to beta-lactamase antibiotics which include the Penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and the Cephalosporins. It is a major concern in hospital settings, mainly because of its easy mode of spread, its resistance to the commonly used antibiotics thus leading to a delayed wound healing, sepsis and a longer hospital stay. Historically, MRSA has been linked to patients in hospitals or nursing home settings, but outbreaks have been reported among previously healthy members of the community, further increasing the awareness of MRSA[2]. Most

**Correspondence*
Dr. Priti Singh
Tutor, Department of Microbiology, Patna Medical College and Hospital, Patna, India.
E-mail: priti23singh@gmail.com

transmission of MRSA from patient to patient is thought to be mediated by transiently colonised healthcare workers, although airborne dispersal and transmission through contacts with contaminated surfaces may also be important. Attempts to control this spread have relied principally on three measures: hand hygiene among healthcare workers,restriction of antibiotics, and the detection and isolation of infected or colonized patients[3].The correct detection of MRSA plays a crucial role in the treatment and also in the prevention of further spread of the strain in the hospital. There are several tests such as oxacillin agar screening test, Oxacillin disk diffusion, broth micro dilution and rapid tests such as latex agglutination MRSA screen test, rapid ATB Staph and automated Viteksystem. These differ in sensitivity and specificity. It is clinically crucial to determine rapidly whether S. aureus isolates are methicillin resistant or not, as this is very important for both treatment and requires extensive hygienic precautions tolimit the spread of such strains4 Methicillin resistance in S.aureus is associated with the production of an altered penicillin-binding protein, PBP2a, encoded by the mec gene complex[5,6]. Genotypic tests involving detection of mecA gene by polymerase chain reaction (PCR) are the preferred methods[7,8] but they are not practical for routine use in many clinical laboratories.But the wide prevalence of the MRSA infections has made it important to have an ideal method for its detection. This method should not only be reliable butalso cheap and time saving. It should be possible to use this method even in the remote areas of the country. Hence this study was undertaken to compare three conventional methods namely oxacillin disk susceptibility, cefoxitin disk diffusion and the oxacillin screen agar with the gold standard MIC(Minimum inhibitory concentration) determination in terms of their sensitivity and specificity.

Material and methods

This study was conducted in Department of Microbiology, at Patna Medical College and Hospital, Patna from March 2020 to September 2020. 75 strains of Staphylococcus aureus were included in the study. The strains were confirmed by slide and tube coagulase tests carried out using standard methodology.Ethical clearance was obtained from the institutional ethical and research committee.

All the tests were performed according to the Clinical Laboratory Standards Institute(CLSI)Oxacillin screen agar: Oxacillin screen agar was performed by direct colony suspension method and adjusted to match 0.5 McFarl and turbidity standards. The suspension was inoculated on Mueller-Hinton agar containing 4% NaCl and with 6µg/ml Oxacillin. Plates were then incubated 24 hours at 35°C. Any growth on the plate containing Oxacillin was considered as resistant to methicillin.

Oxacillin disk diffusion

Oxacillin disk susceptibility testing was performed according to Clinical Laboratory Standards. Briefly a bacterial suspension adjusted to 0.5 McFarl and was inoculated onto Mueller-Hinton agar. A filter paper disk containing 1µg oxacillin was placed on the inoculated Mueller Hinton agar. Plate was incubated in 35°C for 24 hours.The diameter of zone of inhibition was measured. If the zone of inhibition is less than 13, the strain is resistant. Cefoxitin disk diffusion: Briefly a bacterial suspension adjusted to 0.5 McFarland was inoculated onto Mueller- Hinton agar. Cefoxitin disk diffusion test was performed using 30 microgram disk and zone sizes were measured. If the zone of inhibition is less than 20, the strain is resistant.

Minimum inhibitory concentration determination

Agar dilution method was used to determine the Minimum Inhibitory Concentration (MIC).It is a quantitative method for determining the minimum inhibitory concentration of the antibiotics. The required dilutions of the antibiotics were made as follows: Stock solution containing 2000µg/ml of the antibiotic to be tested was prepared. The solutions were diluted to 0.5µg/ml, 1µg/ml, 2µg/ml, 4µg/ml, 8µg/ml, 16µg/ml, 32µg/ml and 64µg/ml using standard methodology. These antibiotic solutions were then added to molten Mueller Hinton agar and allowed to set. Thus, Mueller Hinton agar plates of varying antibiotic concentrations were prepared. A control plate without any antibiotic was also prepared. The organisms were inoculated into nutrient broth and incubated for 3-4 hours and turbidity was adjusted to 0.5Macfarlands standard. They were spot inoculated onto the surface of the medium and incubated at 35°C for 16-18 hrs. After incubation the readings were taken. The control plates without antibiotic were checked for the growth and then the test plates were read. The concentration at which growth is completely inhibited was considered as the MIC. Organisms with MIC of 4 and above were considered as MRSA and those with MIC below 4 were considered MSSA. The organisms were then reported as sensitive, intermediate or resistant based on the MIC cut off.

Results

This study was conducted with 75 strains of Staphylococcus aureus. The three conventional methods namely the oxacillin screen agar, cefoxitin disk diffusion and oxacillin disk diffusion were done.

MIC determination was also done. These conventional methods were then compared with MIC determination. Their sensitivity and specificity were calculated. MIC determination was taken as the gold standard.The total number of organisms with MIC at different antibiotic concentrations was calculated (Table 1). It was found that 5 (7%) of organisms showed an MIC of 64µg/ml. However, a maximum of 27 (36%) organisms showed an MICof 32µg/ml. While the number of organisms with MIC</=0.5, 1,2,4,8 and 16 were 6(8%), 2 (3%), 11(14%), 6(8%),8(11%) and 10(13%) respectively. Among the MSSA it was found that around 11 out of 19 (58%) showed a higher range MIC of 2.

Table 1: MIC values and susceptibility pattern of staphylococcus aureus strains

Minimum inhibitory concentration value (µg/ml)	Number of Staphylococcus aureus	Type of Staphylococcus aureus
0.5	6(8%)	MSSA
1	2(3%)	MSSA
2	11(14%)	MSSA
4	6(8%)	MRSA
8	8(11%)	MRSA
16	10(13%)	MRSA
32	27(36%)	MRSA
64	5(7%)	MRSA

Total MSSA=19(25.33%),Total MRSA=56(74.66%)

Out of the 75 strains of Staphylococcus aureus, all the methods detected 19 MSSA (25%) and 56(75%) MRSA. This was in correlation with the results obtained by MIC determination. Thus, it was seen that all the three-methods oxacillin screen agar, cefoxitin disk diffusion and oxacillin disk diffusion showed a 100 percent sensitivity and specificity. (See table 2)

Table 2: Sensitivity and specificity

Name of the test	Number of sensitive strains	Number of resistant strains	Sensitivity	Specificity
Cefoxitin disk screening	19	56	100	100
Oxacillin disk susceptibility	19	56	100	100
Oxacillin screen agar	19	56	100	100
MIC determination	19	56	100	100

Discussion

In recent years, detection of mecA by PCR is considered as the gold standard for identification of MRSA. In this study, they evaluated other methods as alternatives to PCR[9]. In the present study MIC was considered as the gold standard and cefoxitin disc diffusion, oxacillin disc diffusion and oxacillin screen agar were compared. It was seen that all the methods had an equal sensitivity and specificity in the identification of the MRSA.Broekeme et al., reported the sensitivity and specificity of cefoxitin disc diffusion method 97.3% and 100%, respectively among 1,611 S. aureus isolates. However in the present study both sensitivity and specificity of cefoxitin disc diffusion was 100%.[10]In current study, MIC showed the sensitivity and specificity about 100%, respectively. Thiswas similar to the results obtained in a study conducted by Rahbar et al. where sensitivity and specificity were both 100% for MIC strip test[11]. According to a study by Mohammad Reza Pourmand et al cefoxitin disc diffusion has both high sensitivity and specificity as compared with mecA PCR and hence can be a good alternative to molecular methods due to its low cost for clinical laboratories[12]. This was similar to the results obtained in our study that cefoxitin disc diffusion method showed a hundred percent sensitivity and specificity, however we used MIC determination as the gold standard.A study by Priya Datta et al recommend that along with cefoxitin disc diffusion, another method, preferably latex agglutination, should be routinely used in all hospitals to detect MRSA.In our study also we recommend the use of any two methods in combination namely cefoxitin disc diffusion, oxacillin disc diffusion or oxacillin screen agar for a reliable result.

Conclusion

This study was done on 75 strains of Staphylococcus aureus to determine an accurate method for the

detection of MRSA. The three conventional methods namely cefoxitin disk susceptibility, oxacillin disk diffusion and oxacillin screen agar were done and then compared with minimum inhibitory concentration determination, which was considered as the gold standard.

The results as obtained by cefoxitin disk susceptibility, oxacillin disk diffusion and oxacillin screen agar correlated well with that obtained by MIC determination and was found to be equally sensitive and specific. However, for better reliability of the result, we recommend that any two of these methods be used in a combined way for accuracy

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